AD NUMBER

AD842906

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

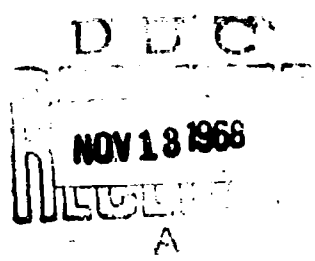
Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; 15 FEB 1968. Other requests shall be referred to the Army Biological Laboratory, Attn: Technical Releases Branch [TID], Fort Detrick, MD 21701.

AUTHORITY

SMUFD, per d/a ltr, 15 Feb 1972

TRANSLATION NO. 2137

DATE: 15 February 1968



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

12

APPLICATION OF THE FLUORESCENT ANTIBODY TECHNIQUE TO DRIED AND ELUTED BLOOD: A COMPARISON WITH FTA, TPI AND LIPOIDIC ANTIGEN TESTS OF THE SERUM

La Prophylaxie Sanitaire et Morale (Moral and Health Prophylaxis)
Vol 35, No 9, 1963
Pages 222 - 230

A. Vaisman,
A. Hamelin,
T. Guthe and
L. Descombes
(Technical collaboration)

The immuno-fluorescence test for the serologic diagnosis of syphilis (FTA, fluorescent treponemic antibody test) is now well-known. Since its introduction by Deacon, Falcone and Harris (1957), the method has been studied by a number of research workers who have confirmed its utility in the specific diagnosis of syphilis: (Borel/Durel, 1959; Censuales and Garofalo, 1959; Fife et al., 1959; Fribourg-Blanc and Niel, 1962; Montgomery, Surland and Knox, 1960; Niel and Fribourg-Blanc, 1962; Nielson and Idsoe, 1963; Olansky and McCormick, 1960; Pillot and Borel, 1961; Thivolet, Grospiron and Mourat, 1960; Vaisman and Hamelin, 1961; Wilkinson, 1961).

The FTA test is second only to the TPI test (the treponema immobilization test of Nelson and Mayer) among the sero-diagnostic tests for treponematosis in its sensitivity, specificity and certainty.

With FTA a reproducibility of about 95% (reacting serums/non-reacting serums) was obtained by the majority of laboratories participating in the collective trials recently organized by the WHO in Denmark, France, Japan,

Great Britain, and the U.S.A., with the participation of the Chamblee Laboratory (Center for Communicable Diseases, United States Public Health Service, Atlanta, Georgia) acting as WHO reference laboratory.

The Fournier Institute, a participant in the evaluation, has used the FTA method for more than four years, this method having been compared with the other treponemic and lipoidic antigen tests. Certain technical details, as well as the results obtained, were the subject of a preceding article (Vaisman and Hamelin, 1961).

The principle of the FTA test lies in making apparent the antigen-antibody complex with the aid of a fluorescent antiglobulin corresponding to the serum of the species. This antiglobulin is fixed on the antibody which attaches itself to the treponemas on a slide. In the absence of antibodies, the antiglobulin is not fixed and the treponemas remain non-fluorescent.

As a participant in the World Health Organization research program upon treponematoses, the Fournier Institute is studying the possibility of testing FTA upon samples of eluted dried blood, obtained by pricking of a finger. This method, which avoids venous puncture, is simple and rapid and may be used for the examination of a large number of individuals during collection studies of venereal syphilis or of endemic treponematoses (pian, endemic syphilis, pinta). Our research is also helping to aid in the dispatch of blood samples to laboratories, especially in the developing countries where transportation from the sampling site may often be long and difficult. One method capable of coping with the difficulties of travel consists of absorbing whole capillary blood upon a medium from which it may be easily eluted when needed for testing. The use of blood dried on filter paper has already been tried (Chediak, 1932) for lipoidic antigen tests (Ko-Da-Guo, 1938). Certain workers (Demanche, 1940; Ko-Da-Guo, 1938) achieved encouraging results, whereas others (Karim, 1954; Harris and Olansky, 1951; Hogan and Bush, 1950) encountered difficulties, probably due to differences in the filter papers and to the presence in the paper of certain chemicals which interfere with the seroreaction during elution. For this reason we used, in our first studies of the FTA technique, blotting paper (Canson), chromatographic and electrophoresis paper (Arches) and ultra-filtrating

membranes (Millipore). However, only the blotting paper, Canson no. 435, used previously by Demanche, proved satisfactory. The results obtained with the first series of eluates and of serums subjected to the FTA test are given below, along with an outline of the conditions under which the tests were performed; for comparison with sera removed from the same patients by venous puncture, the TPI and the classic lipoidic antigen tests were performed, the results being given also.

TECHNIQUE

Canson no. 435 Blotting Paper:

According to the manufacturer, this is a rag paper weighing 0.25 kg/m^2 which does not contain substances soluble in water. Ash content is 1.5%. Discs (roundels) 15 mm in diameter were cut from the paper using a paper-punch.

Withdrawal of blood:

The blood was obtained by pricking a finger and directly absorbing the blood until the roundels were completely soaked. Preliminary studies have shown that the blotting paper discs retain an average of 0.1539 gm of blood, which corresponds to 0.0846 gm of plasma. The discs were dried in the open air for 1 to 2 hours. When completely dry they were kept in a small plastic envelope prior to the FTA test.

Preparation of the FTA test:

The dry disc was immersed in 8 ml of phosphate-buffer solution, pH 7.2, for two hours, which corresponds to a dilution of fresh plasma of 1/100. The disc loses its color completely, the eluate being a brownish-red. The latter may be used for the FTA in the same manner as the normal 1/100 serum in accordance with the technique habitually used by the Fournier Institute.

RESULTS

For purposes of comparison, besides the normal

samples removed from the same individuals by venous puncture, all blood samples removed by finger-pricking were taken on discs in duplicate. Besides the FTA and TPI, other tests were carried out on the sera: Kolmer cardiolipin complement fixation, Reiter antigen complement fixation as well as Kahn and Kline flocculation tests. The eluate of one of the roundels was subjected to FTA at the same time as the serum stemming from the venous puncture; the other disc was held at a temperature of 20-25° for a period varying from several days to two months, to allow study of possible deterioration of the antibodies and therefore a possible variation of the results of the tests.

The present, preliminary, study involves 150 samples of differing reactivity. The results obtained with FTA100 upon the eluates of the discs and on the sera removed by venous puncture are compared in Table 1.

It is important to analyze the variations of the FTA_{100} tests in detail according to whether they were carried out on eluted blood or on serum. Complete agreement was observed in 111 cases (74%), greater sensitivity in 23 cases, lower sensitivity in 16 cases. However, in all cases, this difference did not exceed plus or minus one scale division. This variation is less than that observed when the same serum is examined several times by the same observer or by different observers. In all-or-nothing terms (reacting or non-reacting sera), agreement was found in 144 cases (96%). Further, several quantitative examinations of sera and of eluates (discs) gave identical titres. One may therefore conclude that the results obtained with the FTA_{100} technique on blood dried on discs of Canson no. 435 blotting-paper (finger-pricking) are practically identical to results obtained using the same technique on sera removed by venous puncture.

Table 2 summarizes the results of the FTA, TPI and the lipoid tests of sera obtained by venous puncture. The following observations are of note:

a. The positive TPI tests belonged, without exception, to the 16 sera giving a ++++ reaction, while the lipoid tests were negative in one case. This involved a long-standing case of syphilis which had been treated but was negative to the classic tests.

TABLE 1 - Comparison between FTA_{100} tests on eluates of blood dried upon discs of blotting paper and FTA_{100} tests on serum removed by venous puncture from the same individuals

FTA_{100} (serum removed by venous puncture)	FTA_{100} (eluted dried blood removed by finger pricking)						
	++++	+++	++	+	+	-	Total
++++	12	4	-	-	-	-	16
+++	2	27	2	-	-	-	31
++	-	8	19	1	-	-	28
+	-	-	5	15	3	5	28
+	-	-	-	2	2	1	5
-	-	-	-	4	2	36	42
Total	14	39	26	22	7	42	150

Total agreement	111/150 = 74%
Stronger reaction in 23 cases . . .	23/150 = 15%
Weaker reaction in 16 cases	16/150 = 11%
Agreement within one scale division .	150/150= 100%
All-or-nothing agreement . . . . . (reacting or non-reacting)	144/150= 96%

b. Of 31 sera giving a +++ reaction to FTA, 30 gave positive TPI's and one negative; this serum came from a case of primary syphilis. The 31 sera reacted to the lipoid tests.

c. Of the 28 sera giving a ++ reaction to FTA, 25 gave positive TPI's and 3 negative TPI's; these three

sera were negative to the lipoid tests also. They involved sera from cases of "pre-serologic" syphilis with primary chancres, which shows the precocious character of the reactivity to FTA compared with the other serologic tests.

The three groups of sera mentioned above which gave the positive results ++++, +++ and ++ were considered as reacting, while those giving reactions +, + and zero were considered as non-reacting.

d. From among the 28 sera that were non-reacting, but + to FTA, 5 TPI positives were found. These sera came from long-standing, treated cases of syphilis with reactivation of the lipoid tests, the TPI reactivity being, however, irreversible. Furthermore, three sero-reactions to lipoidic antigen were probably false positive reactions because the TPI was negative for the same three subjects and no syphilitic antecedents were known to any of the three.

e. The five non-reacting sera, + to FTA, gave one negative TPI, but two reacted to lipoid tests. False sero-reactions in subjects without known syphilitic antecedents are probably involved here.

f. Only one positive TPI was observed among the 42 sera which had not caused immunofluorescence. This involved a long-standing, treated case of syphilis. Furthermore, another serum reacted to the classic lipoid tests but gave a negative TPI; there were no known syphilitic antecedents.

A prolonged positive TPI reaction for long-standing, treated cases of syphilis which are, however, negative to lipoid tests has therefore been noticed on several occasions, as during the preceding research. Similarly, the finding of increased sensitivity of TPI with respect to FTA has been confirmed in the case of long-standing, untreated cases of syphilis (Eng., Nielsen and Wereide, 1963).

Table 3 summarizes the FTA_{100} obtained with eluted dried blood, and compares them with the TPI and with lipoid tests applied to sera obtained by venous puncture.

TABLE 2 - Results of the FTA, the TPI and the lipoidic antigen tests used for serums obtained by venous puncture

FTA	Number of Sera	TPI		Lipoid Tests	
		Reacting	Non-Reacting	Reacting	Non-Reacting
	reacting				
++++	16	16	0	15	1
+++	31	30	1	31	0
++	28	25	3	25	3
	non-reacting				
+	28	5	23	3	25
+	5	0	5	2	3
-	42	1	41	1	41

TABLE 3 - Comparison between FTA applied to eluted dried blood, TPI and the lipoid tests applied to sera obtained by venous puncture

FTA	Number of Eluates	TPI		Lipoid Tests	
		Reacting	Non-Reacting	Reacting	Non-Reacting
	reacting				
++++	14	14	0	13	1
+++	39	38	1	39	0
++	26	22	4	22	4
	non-reacting				
+	22	2	20	1	21
+	7	0	7	1	6
-	42	1	41	1	41

Comparison with Table 2 permits one to observe that only minimum differences of sensitivity exist between the FTA serum and FTA disc techniques as compared with TPI and classic serology. In Table 2, 4 sera non-reactive to TPI and reactive to FTA are to be found, this discordance increasing to 5 in Table 3. For the non-reactive FTA sera the number of anomalous results is 6, whereas there are only 3 TPI reactive sera in comparison with the FTA discs. In sum, there are, therefore, 10 TPI anomalies compared with the FTA serum and only 8 TPI anomalies compared to the FTA discs.

Maintenance of reactivity during storage of the blotting paper:

As we have indicated above, all the samples of blood obtained by finger-pricking were prepared in duplicate. The first blotting-paper roundel was examined at the same time as the serum, whereas the other was kept at a temperature of 20-25°C for a period of up to 60 days. Of the 150 stored discs, 40 (26.7%) have been examined to date; in no case has a loss of reactivity been observed. The fact that the observed differences have never exceeded plus or minus onescale division must be attributed to the reproducibility of the FTA technique rather than to the properties of antibody conservation during storage. This conclusion seems to be confirmed by the fact that, in some cases, an increased reactivity was observed, even after 60 days of storage.

The study of conservation under different environmental conditions continues as does investigation of storage at temperatures above 25°C. We are also studying the storage of discs which have travelled by air under diverse climatic and other conditions. The results of these studies will form the subject of further communications.

CONCLUSIONS

Past experiments which have used filter paper for lipidic tests upon blood drawn from the fingertips, the sample being first dried then eluted, have not been successful; however, better results have been obtained using blotting paper as the absorbing medium. With the introduction of the immuno-fluorescence technique into the serology

of syphilis and other treponematoses, it appeared useful to try the FTA test under these conditions using blotting paper as the absorbing medium: it is of great interest to perfect a simple method usable in regions where one must collect serologic data for the study of treponematoses, and cannot obtain blood samples by venous puncture. The present article describes the preliminary results obtained with FTA using discs of Canson (no. 435) blotting paper. After absorption, drying and storage, 150 eluted samples taken from both normal and infected subjects were tested by FTA. We studied, in parallel, the reactivity to FTA, TPI and to lipoid tests of sera obtained from the same subjects by venous puncture. The variations of sensitivity, of specificity and of reproducibility noted in the discs used in the FTA_{100} technique were insignificant; the results were practically identical to those of FTA_{100} used on sera obtained by venous puncture. No deterioration of the antibody was observed as a result of storage of discs at 20-25°C for periods ranging from several days up to 60 days. In certain cases, the precocious appearance of fluorescent antibodies was noted in the sera of primary syphilis, preceding those of the immobilisins and reagins. Comparative serologic examinations of old, treated syphilitic infections have, on the other hand, confirmed that the sensitivity of TPI is superior to that of FTA (and of the serology of classic reagins) in these particular cases.

The advantages of the finger-pricking method of withdrawal are evident. Furthermore, discs of blotting paper impregnated with dried blood can be mailed very conveniently to the FTA testing laboratory. This method eliminates all risk of hemolysis, infection, breakage, etc., inherent in the mailing of ampoules or of glass flasks.

The results of studies on the behavior of fluorescent antibodies at temperatures above 25°C to which they may be exposed during storage, transportation, etc., will be published in the future.

/Authors A. Vaisman and A. Hamelin and technical collaborator L. Descombes are from the Experimental Serology and Chemotherapy Laboratory, Alfred Fournier Institute, Paris, France, and author T. Guthe is from the Communicable Diseases Section, World Health Organization, Geneva, Switzerland./

BIBLIOGRAPHY

Borel, L. J. and Durel, P. Path. et Biol., 7, 2317; 1959.

Censuales, S. and Garofalo, V. Riv. Ist. sieroter ital., 34, 161; 1959.

Chediak, A. Rev. med. cuba., 43, 947; 1932.

Deacon, W. E., Falcone, V. H. and Harris, A. Proc. Soc. exp. Biol. (N.Y.), 96, 477; 1957.

Demanche, R. Presse med., 48, 669; 1940.

Eng, J., Nielsen, H. Aa. and Vereide, K., Bull. Org. mond. Sante, 28, 533; 1963.

Fife, E. H., Bryan, B. M., Sanders, R. W. and Muschel, L. H. Amer. J. clin. Path., 36, 105; 1961.

Fribourg-Blanc, A. and Niel, G. Presse med., 41, 1875; 1962

Karim, M. A., Indian J. Derm. Venereol., 20, 59; 1954.

Ko-Da-Guo Dtsch. med. Wschr., 19, 575; 29, 1035; 1938.

Harris, A. and Olansky, S. J. vener. Dis. Inform. 32, 1; 1951.

Hogan, R. B. and Busch, S. J. vener. Dis. Inform. 31, 37; 1950.

Montgomery, C. H., Surland, J. and Knox, J. M. J. invest. Derm., 35, 95; 1960.

Niel, G. and Fribourg-Blanc, A. Ann. Inst. Pasteur, 102, 616; 1962.

Nielsen, H. Aa. and Idsoe, O. Acta path. microbiol. scand. (in press); 1963.

Olansky, S. and McCormick, G. E. Arch. Derm., 81, 59; 1960.

Pillot, J. and Borel, L. J. C. R. Acad. Sci (Paris), 252, 954; 1961.

Thivolet, J., Grospiron, D. and Mourat, M. Rev. Hyg. Med. soc., 8, 501; 1960.

Vaisman, A. and Hamelin, A. Presse med. 69, 1157; 1961.

Wilkinson, A. E. Brit. J. vener. Dis., 37, 59; 1961.